

The effective ligase activity was determined by comparing the rate of circularization of EcoRI linerized pMS19 in the solvent above compared with ligase buffer, where 50% of the molecules are circularized in 25 minutes at 100 U/ml. The ligase activity determined was approximately 0.5 U/ml.

Figure 7 depicts the a fluorogram of the branch migration experiments. After 30 minutes, 50% of the recipient duplexes have captured a linker in the presence of BT-D-MedC-1. The rate of linker capture with BT-D-MedC-1 was greater than thirty times that predicted for the ligase activity and the concentrations of displacer-linker duplex using the method in Example 8.

The gel was dried for autoradiography. The branch migration products visible in the fluorogram in lanes 4-6 were visible in the autoradiogram, demonstrating that the new fluorogram bands represent branch migration-mediated capture products.

After overnight incubation, branch migration products could be seen with both displacers with 50% yield of ligated product with BO-D-MedC-1. Thus, the inclusion of a particular, unmodified, triplex-forming region on displacer BT-D-MedC-1, but not BO-D-MedC-1, stabilized the branch migration intermediates by greater than 30-fold.

Although the foregoing invention has been described in some detail by way of illustration and example for clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the following claims.

## CLAIMS

We claim:

1. In a method of forming a non stable complex between one strand of a recipient polydeoxyribonucleotide sequence and a displacer sequence of single stranded DNA where the displacer sequence is at least partially

1 complementary to such strand of a recipient polydeoxynucleotide  
2 sequence, the improvement comprising stabilizing the complex.  
3

4 2. The method of claim 1 wherein the nonstable complex is stabilized  
5 by the presence of at least one modified nucleotide in the displacer  
6 strand.  
7

8 3. The method of claim 1 wherein the complex is stabilized by  
9 forming a DNA triplex between the displacer sequence and the  
10 recipient duplex.  
11

12 4. The method of claim 1 wherein the displacer strand comprises a  
13 nucleotide sequence and a sequence specific DNA binding moiety that  
14 does not significantly melt the recipient DNA duplex at the site to  
15 which it attaches.  
16

17 5. The method of claim 1 wherein the displacer sequence is  
18 duplexed with a linker in a displacer-linker duplex wherein  
19 the displacer-linker duplex comprises two strands;  
20

21 1. a displacer strand of which a portion comprises nucleotides  
22 complementary to one strand of a recipient polydeoxynucleotide  
23 duplex and a portion which comprises a sequence complementary to  
24 and hybridized with a linker strand, and  
25

26 2. a linker strand complementary to and hybridized with the  
27 displacer strand.  
28

29 6. An oligo- or polydeoxynucleotide displacer-linker duplex which is  
30 capable of initiating branch migration at the end of a recipient  
31 polydeoxynucleotide duplex without the prior formation of a stable  
32 hybrid with such recipient polydeoxynucleotide duplex, which  
33 displacer-linker duplex comprises two strands;  
34

35 a. a displacer strand of which a portion comprises nucleotides  
36 complementary to one strand of a recipient polydeoxynucleotide  
37 duplex and a portion which comprises a sequence complementary to  
38 and hybridized with a linker strand, and  
39

1                   b. a linker strand complementary to and hybridized with the  
2                   displacer strand.

3

4                   7. The displacer-linker duplex of claim 6 which can initiate branch  
5                   migration at a restriction endonuclease cleavage site.

6

7                   8. The displacer-linker duplex of claim 6 which can hybridize to and  
8                   initiate branch migration adjacent to a 3' or 5' single stranded  
9                   extension on the recipient polydeoxynucleotide duplex.

10

11                  9. The displacer-linker duplex of claim 6 wherein at least one of the  
12                  nucleotides complementary to one strand of the recipient  
13                  polydeoxynucleotide duplex is modified to increase the stability of the  
14                  hybrid displacer-recipient duplex.

15

16                  10. The displacer-linker duplex of claim 6 wherein at least one of the  
17                  nucleotides complementary to one strand of a recipient  
18                  polydeoxynucleotide duplex is modified to increase the melting  
19                  temperature of the hybrid displacer-recipient duplex.

20

21                  11. The displacer-linker duplex of claim 9 wherein the modified  
22                  nucleotide is selected from the group consisting of modified  
23                  nucleotides which increase the association constant with the  
24                  complementary deoxynucleotide by at least about 20 percent.

25

26                  12. The displacer-linker duplex of claim 9 wherein the modified  
27                  nucleotide is selected from the group consisting of 5-halogenated  
28                  pyrimidine nucleotides, 5-methyldeoxycytidine, diaminopurine  
29                  deoxynucleotide, ribonucleotides, and 2'-alkylated ribonucleotides.

30

31                  13. The displacer-linker duplex of claim 9 wherein the modified  
32                  nucleotide is a 5-halogenated pyrimidine nucleotide.

33

34                  14. The displacer-linker duplex of claim 9 wherein the modified  
35                  nucleotide is 5-bromodeoxyuridine.

36

1 15. The displacer-linker duplex of claim 9 wherein the modified  
2 nucleotide is 5-methyldeoxycytidine.

3

4 16. The displacer-linker duplex of claim 6 which contains a  
5 modification which permits detection of the displacer-recipient  
6 hybrid.

7

8 17. The modified displacer-linker duplex of claim 16 wherein the  
9 modification is selected from the group consisting of radioactive  
10 labels, fluorescent labels and targets for detection, including biotin  
11 moieties, enzymes and phosphorothioate linkages.

12

13 18. The modified displacer-linker duplex of claim 16 wherein the  
14 modification is present in the linker.

15

16 19. The displacer-linker duplex of claim 6 which contains a  
17 modification which allows capture of the displacer-recipient hybrid by  
18 affinity chromatography.

19

20 20. The displacer-linker duplex of claim 19 wherein the modification  
21 is selected from the group consisting of biotin moieties and  
22 phosphorothioate linkages.

23

24 21. The displacer-linker duplex of claim 19 wherein the modification  
25 is present in the linker.

26

27 22. The displacer-linker duplex of claim 6 which also comprises a 5'  
28 or 3' single-stranded extension complementary to a 5' or 3' single-  
29 stranded extension resulting from the digestion of a  
30 polydeoxynucleotide duplex with a restriction endonuclease.

31

32 23. An artificially constructed polydeoxynucleotide hybrid comprising  
33 a naturally occurring recipient polydeoxynucleotide duplex hybridized  
34 to the displacer-linker duplex of any of claims 6-22.

35

36

1           24. The artificially constructed polydeoxynucleotide hybrid of any of  
2           claims 6-22 wherein the linker strand is covalently linked to one of  
3           the strands of the recipient duplex.

4

5           25. An artificially constructed polydeoxynucleotide hybrid comprising  
6           a naturally occurring recipient polydeoxynucleotide duplex hybridized  
7           to the displacer of claims 57-87.

8

9           26. A method of modifying a recipient polydeoxynucleotide duplex by  
10          contacting such recipient polydeoxynucleotide duplex with a  
11          displacer-linker duplex under conditions that permit the formation of  
12          a hybrid polydeoxynucleotide duplex, wherein  
13            the displacer-linker duplex comprises two strands;  
14            1. a displacer strand of which a portion comprises  
15            nucleotides complementary to one strand of a recipient  
16            polydeoxynucleotide duplex and a portion which comprises a  
17            sequence complementary to and hybridized with a linker strand, and  
18            2. a linker strand complementary to and hybridized with  
19            the displacer strand.

20

21          27. The method of claim 26 wherein the recipient duplex terminates  
22          in a 3' or 5' single stranded extension and the displacer strand  
23          contains a sequence complementary to the extension.

24

25          28. The method of claim 26 where the hybrid is stabilized after  
26          formation of the hybrid polynucleotide duplex.

27

28          29. The method of claim 28 wherein the hybrid is stabilized by  
29          covalently linking the linker strand of the displacer-linker duplex to  
30          the strand of the recipient duplex complementary to the displacer  
31          strand.

32

33          30. The method of claim 29 wherein the covalent link is a  
34          phosphodiester linkage.

35

36

1      31. The method of claim 29 wherein the hybrid is stabilized by  
2      ligating the linker strand of the displacer-linker duplex to the strand  
3      of the recipient duplex complementary to the displacer strand using  
4      T4 DNA ligase.

5

6      32. The method of claim 26 wherein at least one of the nucleotides  
7      complementary to one strand of the recipient polydeoxynucleotide  
8      duplex is a modified nucleotide which increases the stability of the  
9      hybrid displacer-recipient duplex.

10

11     33. The method of claim 26 wherein at least one of the nucleotides  
12    complementary to one strand of the recipient polydeoxynucleotide  
13    duplex is a modified nucleotide which increases the melting  
14    temperature of the hybrid displacer-recipient duplex.

15

16     34. The method of claim 32 wherein the modified nucleotide is  
17    selected from the group consisting of modified nucleotides which  
18    increase the association constant with the complementary  
19    deoxynucleotide by at least about 20 percent.

20

21     35. The method of claim 32 wherein the modified nucleotide is  
22    selected from the group consisting of 5-halogenated pyrimidine  
23    nucleotides, 5-methyldeoxycytidine, diaminopurine deoxynucleotide,  
24    ribonucleotides, and 2'-alkylated ribonucleotides.

25

26     36. The method of claim 32 wherein the modified nucleotide is a 5-  
27    halogenated pyrimidine nucleotide.

28

29     37. The method of claim 32 wherein the modified nucleotide is 5-  
30    bromodeoxycytidine.

31

32     38. The method of claim 32 wherein the modified nucleotide is 5-  
33    methyldeoxycytidine.

34

35

36

1       39. The method of claim 32 wherein from about 10 percent to about  
2       80 percent of the nucleotides complementary to one strand of the  
3       recipient polydeoxynucleotide duplex are modified nucleotides.

4  
5       40. A method of labelling an artificially constructed nucleic acid  
6       hybrid of a naturally occurring recipient polydeoxynucleotide duplex  
7       hybridized to a displacer-linker duplex which is capable of initiating  
8       branch migration at the end of the recipient polydeoxynucleotide  
9       duplex without the prior formation of a stable hybrid with such  
10      recipient polydeoxynucleotide duplex, which displacer-linker duplex  
11      comprises two strands;

12       1. a displacer strand of which a portion comprises nucleotides  
13       complementary to one strand of a recipient polydeoxynucleotide  
14       duplex and a portion which comprises a sequence complementary to  
15       and hybridized with a linker strand, and

16       2. a linker strand complementary to and hybridized with the  
17       displacer strand,

18       which method of labelling comprises modifying the displacer-linker  
19       duplex to incorporate therein a modification which permits detection  
20       of the artificially constructed nucleic acid hybrid.

21  
22       41. The method of claim 40 wherein the displacer-linker duplex is  
23       modified prior to hybridization with the naturally occurring recipient  
24       polydeoxynucleotide duplex.

25  
26       42. The method of claim 40 wherein the linker strand of the  
27       displacer-linker duplex is covalently linked to the strand of the  
28       naturally occurring recipient polydeoxynucleotide duplex  
29       complementary to the displacer strand.

30  
31       43. The method of claim 40 wherein the modification is selected  
32       from the group consisting of radioactive labels, fluorescent labels,  
33       enzymes and chemical labels including biotin moieties and  
34       phosphorothioate linkages.

1       44. The method of claim 40 wherein the modification is selected  
2       from the group consisting of targets for affinity chromatography.  
3  
4       45. The method of claim 44 wherein the modification is selected  
5       from the group consisting of biotin moieties and phosphorothioate  
6       linkages.  
7  
8       46. The method of claim 40 wherein the modification comprises a 5'  
9       or 3' single-stranded extension of the displacer-linker duplex, which  
10      extension  
11       1) is unaffected by formation of the displacer-linker-recipient  
12       polydeoxynucleotide duplex hybrid, and  
13       2) is a target for attachment to polydeoxynucleotide duplexes  
14       containing complementary 5' or 3' single stranded extensions.  
15  
16      47. The method of claim 40 used to label one end of a cloned  
17       deoxynucleotide insert in a vector.  
18  
19      48. The method of claim 47 where the vector is a plasmid vector.  
20  
21      49. The method of claim 47 where the vector is a cosmid vector.  
22  
23      50. The method of claim 47 where the vector is a yeast artificial  
24       chromosome vector.  
25  
26      51. In a method of restriction endonuclease mapping of an insert, the  
27       improvement comprising labelling the insert by the method of claim  
28       40.  
29  
30      52. In a method of capture of an artificially constructed nucleic acid  
31       hybrid by affinity chromatography, the improvement comprising  
32       labelling the hybrid by the method of claim 40.  
33  
34      53. In a method of enriching a recipient polydeoxynucleotide duplex  
35       in a population of polydeoxynucleotide duplexes, the improvement  
36

1 comprising labelling the recipient polydeoxynucleotide duplex by the  
2 method of claim 40.

3  
4 54. In a method of covalently attaching a restriction endonuclease  
5 linker onto a recipient polydeoxynucleotide duplex, the improvement  
6 comprising labelling the resulting hybrid by the method of claim 40.

7  
8 55. In a method of selectively cloning a recipient polydeoxynucleotide  
9 duplex by covalently attaching a restriction endonuclease linker onto  
10 such recipient polydeoxynucleotide duplex, the improvement  
11 comprising labelling the hybrid by the method of claim 40.

12  
13 56. In a method of isolating clones of contiguous  
14 polydeoxyribonucleotide duplexes by covalently attaching a restriction  
15 endonuclease linker onto the duplexes, the improvement comprising  
16 labelling the clones by the method of claim 40.

17  
18 57. An oligo- or polydeoxynucleotide displacer which is capable of  
19 binding to a recipient polydeoxynucleotide duplex which displacer  
20 comprises

21 1) a first sequence which is capable of initiating triple helix  
22 formation, and which comprises

23 a) at least six consecutive pyrimidine bases or  
24 b) at least seven bases where at least six of the bases are  
25 pyrimidine bases and the seventh base is guanine, and

26 2) a second sequence proximate to such first sequence which is  
27 complementary to and runs antiparallel to the second strand of the  
28 recipient duplex and which is capable of initiating branch migration  
29 proximate to the triple helix.

30  
31 58. The displacer of claim 57 wherein the second sequence is  
32 adjacent to the first sequence.

33  
34 59. The displacer of claim 57 wherein the second sequence is  
35 separated from the first sequence by from 1 to 5 intervening moieties.

1 60. The displacer of claim 59 wherein the intervening moieties are  
2 nucleotides.

3

4 61. The displacer of claim 60 wherein at least one of the moieties is a  
5 modified nucleotide.

6

7 62. The displacer of claim 59 wherein wherein one of the  
8 intervening moieties has an intercalating agent covalently attached.

9

10 63. The displacer of claim 57 wherein at least one of the nucleotides  
11 complementary to one strand of the recipient polydeoxynucleotide  
12 duplex is modified to increase the stability of the displacer-recipient  
13 complex.

14

15 64. The displacer of claim 57 wherein at least one of the nucleotides  
16 complementary to one strand of a recipient polydeoxynucleotide  
17 duplex is modified to increase the melting temperature of the  
18 displacer-recipient complex.

19

20 65. The displacer of claim 63 wherein the modified nucleotide is  
21 selected from the group consisting of modified nucleotides which  
22 increase the association constant with the complementary  
23 deoxynucleotide by at least about 20 percent.

24

25 66. The displacer of claim 63 wherein the modification is in the first  
26 sequence.

27

28 67. The displacer of claim 66 wherein the modified nucleotide is a 5-  
29 halogenated pyrimidine nucleotide.

30

31 68. The displacer of claim 66 wherein the modified nucleotide is  
32 selected from the group consisting of 5-bromodeoxyuridine and 5-  
33 methyldeoxycytidine.

34

35 69. The displacer of claim 63 wherein the modification is in the  
36 second sequence.

1  
2 70. The displacer of claim 69 wherein the modified nucleotide is  
3 selected from the group consisting of 5-halogenated pyrimidine  
4 nucleotides, 5-methyldeoxycytidine, diaminopurine deoxynucleotide,  
5 ribonucleotides and a 2'-alkylated ribonucleotides.

6  
7 71. The displacer of claim 69 wherein the modified nucleotide is a 5-  
8 halogenated pyrimidine nucleotide.

9  
10 72. The displacer of claim 69 wherein the modified nucleotide is 5-  
11 bromodeoxyuridine.

12  
13 73. The displacer of claim 69 wherein the modified nucleotide is 5-  
14 methyldeoxycytidine.

15  
16 74. The displacer of claim 57 which further comprises at least one  
17 moiety attached to a terminus of the oligo or polydeoxynucleotide,  
18 which moiety confers endonuclease resistance to the terminus to  
19 which it is attached.

20  
21 75. The displacer of claim 74 wherein the moiety is attached to the  
22 deoxyribose moiety of a terminal nucleotide.

23  
24 76. The displacer of claim 75 wherein the moiety is indirectly  
25 attached to the deoxyribose moiety of a terminal nucleotide.

26  
27 77. The displacer of claim 74 wherein the moiety is attached to the  
28 hydroxyl group of a terminal nucleotide.

29  
30 78. The displacer of claim 74 wherein the moiety is attached to the  
31 phosphate moiety of a terminal nucleotide.

32  
33 79. The displacer of claim 74 where the moiety is selected from the  
34 group consisting of intercalating agents, isoureas, carbodiimides and  
35 N-hydroxybenzotriazoles.

36

1        80. The displacer of claim 77 wherein the moiety is a  
2        methylthiophosphonate.

3

4        81. The displacer of claim 74 wherein the moiety is a selected from  
5        the group consisting of polypeptides and proteins.

6

7        82. The displacer of claim 74 wherein the moiety is a 2',3'-  
8        dideoxyribose nucleotide attached to the 3'-terminal  
9        deoxyribonucleotide through a phosphodiester linkage.

10

11       83. The displacer of claim 82 wherein the 2',3'-dideoxyribose  
12       nucleotide is a modified 2',3'-dideoxyribose nucleotide.

13

14       84. The displacer of claim 57 which contains a modification which  
15       permits detection of the displacer-recipient hybrid.

16

17       85. The modified displacer of claim 84 wherein the modification is  
18       selected from the group consisting of radioactive labels, fluorescent  
19       labels, enzymes and targets for detection, including biotin moieties  
20       and phosphorothioate linkages.

21

22       86. The displacer of claim 57 which contains a modification which  
23       allows capture of the displacer-recipient hybrid by affinity  
24       chromatography.

25

26       87. The displacer of claim 86 wherein the modification is selected  
27       from the group consisting of biotin moieties and phosphorothioate  
28       linkages.

29

30       88. A method of modifying a recipient polydeoxynucleotide duplex  
31       comprising contacting such recipient polydeoxynucleotide duplex  
32       with the displacer of claim 57 under conditions that permit the  
33       formation of a complex.

34

35       89. The method of claim 88 wherein at least one of the nucleotides  
36       complementary to one strand of the recipient polydeoxynucleotide

1 duplex is modified to increase the stability of the displacer-recipient  
2 complex.

3  
4 90. The method of claim 88 wherein at least one of the nucleotides  
5 complementary to one strand of a recipient polydeoxynucleotide  
6 duplex is modified to increase the melting temperature of the  
7 displacer-recipient complex.

8  
9 91. The method of claim 88 wherein the modified nucleotide is  
10 selected from the group consisting of modified nucleotides which  
11 increase the association constant with the complementary  
12 deoxynucleotide by at least about 20 percent.

13  
14 92. The method of claim 88 wherein the modification is in the first  
15 sequence of the displacer of claim 57.

16  
17 93. The method of claim 92 wherein the modified nucleotide is  
18 selected from the group consisting of 5-bromodeoxyuridine and 5-  
19 methyldeoxycytidine.

20  
21 94. The method of claim 88 wherein the modification is in the  
22 second sequence of the displacer of claim 57.

23  
24 95. The method of claim 94 wherein the modified bases are selected  
25 from the group consisting of 5-halogenated pyrimidine nucleotides, 5-  
26 methyldeoxycytidine, diaminopurine deoxynucleotide, ribonucleotides,  
27 and 2'-alkylated ribonucleotides.

28  
29 96. The method of claim 94 wherein the modified nucleotide is a 5-  
30 halogenated pyrimidine nucleotide.

31  
32 97. The method of claim 94 wherein the modified nucleotide is 5-  
33 bromodeoxyuridine.

34  
35 98. The method of claim 94 wherein the modified nucleotide is 5-  
36 methyldeoxycytidine.

1  
2 99. The method of claim 88 wherein the displacer contains at least  
3 one moiety attached to a terminus of the oligo or polynucleotide,  
4 which moiety confers endonuclease resistance to the terminus to  
5 which it is attached.

6  
7 100. The method of claim 99 wherein the moiety is attached to the  
8 deoxyribose moiety of a terminal nucleotide.

9  
10 101. The method of claim 100 wherein the moiety is indirectly  
11 attached to the deoxyribose moiety of a terminal nucleotide.

12  
13 102. The method of claim 99 wherein the moiety is attached to the  
14 hydroxyl group of a terminal nucleotide.

15  
16 103. The method of claim 99 wherein the moiety is attached to the  
17 phosphate moiety of a terminal nucleotide.

18  
19 104. The method of claim 99 where the moiety is selected from the  
20 group consisting of intercalating agents, isoureas, carbodiimides and  
21 N-hydroxybenzotriazoles.

22  
23 105. The method of claim 101 wherein the moiety is a  
24 methylthiophosphonate.

25  
26 106. The method of claim 99 wherein the moiety is a selected from  
27 the group consisting of polypeptides and proteins.

28  
29 107. The method of claim 99 wherein the moiety is a 2',3'-  
30 dideoxyribose nucleotide attached to the 3'-terminal  
31 deoxyribonucleotide through a phosphodiester linkage.

32  
33 108. The method of claim 107 wherein the 2',3'-dideoxyribose  
34 nucleotide is a modified 2',3'-dideoxyribose nucleotide.

1       109. A method of labelling a displacer-recipient complex comprising  
2       contacting a recipient polydeoxynucleotide duplex with the displacer  
3       of claim 101 under conditions that permit the formation of a complex  
4       wherein the displacer contains a modification which will permit  
5       detection of the displacer-recipient complex.

6

7       110. The method of claim 109 wherein the modification is selected  
8       from the group consisting of radioactive labels, fluorescent and  
9       chemiluminescent labels, enzymes and targets for detection.

10

11      111. The method of claim 109 wherein the modification is selected  
12      from the group consisting of targets for affinity chromatography.

13

14      112. The method of claim 111 wherein the modification is selected  
15      from the group consisting of biotin moieties and phosphorothioate  
16      linkages.

17

18      113. In a method of capture of an artificially constructed nucleic acid  
19      hybrid by affinity chromatography, the improvement comprising  
20      modifying the hybrid by the method of claim 88.

21

22      114. In a method of enriching a recipient polydeoxynucleotide duplex  
23      in a population of polydeoxynucleotide duplexes, the improvement  
24      comprising labelling the recipient polydeoxynucleotide duplex by the  
25      method of claim 88.

26

27      115. In a method for the site specific addition, deletion or alteration  
28      of nucleotides in a recipient polydeoxynucleotide duplex, the  
29      improvement comprising modifying the duplex by the method of claim  
30      88.

31

32      116. In a method of repairing a mutational lesion comprising  
33      replacing a naturally occurring strand of DNA with a modified strand of  
34      DNA, the improvement wherein the new strand is introduced to the  
35      naturally occurring duplex by the method of claim 88 and displaces  
36      the original strand.